

A new species of Tardigrada, *Platicrista ramsayi* sp. nov. from the páramo of Volcán Chiles, Ecuador

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Abstract

A new species of Eutardigrada, *Platicrista ramsayi* sp. nov., is described from moss samples collected at high altitude on Volcán Chiles in the Ecuadorian Andes. This species differs from all others previously described in the genus by having only oblique cuticular bars on the first three pairs of legs. *Platicrista affine* (Mihelčič, 1951) is formally designated as a *nomen dubium*. An amendment to the diagnostic characters for *Platicrista itaquasconoide* is proposed. A key to the species of the genus is presented.

Keywords: Tardigrada, Hypsibiidae, *Platicrista ramsayi* sp. nov., Ecuador, Volcán Chiles, páramo, taxonomy, new species, key to *Platicrista*, *Platicrista affine* (Mihelčič, 1951) *nomen dubium*

Introduction

The genus *Platicrista* Pilato, 1987 was erected during the redescription of the then polyphyletic genus *Diphascon* Plate, 1889 (Pilato 1987). Originally Pilato transferred three species into the new genus: *Platicrista angustata* (Murray, 1905) as the type species; plus *Platicrista affine* (Mihelčič, 1951) and *Platicrista itaquasconoide* (Durante & Maucci, 1975). Two further new species have since been described in the genus, *Platicrista cheleusis* Kathman, 1990 and *Platicrista horribilis* Kaczmarek & Michalczyk, 2003. The former being described from Vancouver Island, Canada and the later from Tehijn Cagan Nuur National Park, Mongolia.

During an ongoing study into high altitude or high latitude tardigrade communities, three specimens of a new species were found in unidentified moss cushions. The mosses were collected from the páramo on Volcán Chiles, on the Ecuadorian side of the international border with Colombia (Fig. 1). Páramos are high altitude ecosystems in South America, typified by nutrient poor soil types, in high rainfall areas above the upper tree-line. The plant communities in this páramo are typified by bunch grasses such as

Calamagrostis effusa and the giant rosette plants *Espeletia pycnophyll* Cuatrec, (Balslev 2001). This research programme was part of wider ecological studies conducted during an expedition from the University of Plymouth, led by Dr. Paul Ramsay, (Ramsay 2001).



FIGURE 1. Map of the location of Volcán Chiles on the international border between Colombia and Ecuador. Map produced by Dr. Paul Ramsay.

Material and methods

The sampling site is identified as station 1 on Figure 2, the most westerly and lowest altitude collecting site of the current study, 3340 m a.s.l. Each sample of moss was placed into either an acid-free manila envelope or wrapped in a page from the local newspaper. The collected sample materials were air dried prior to being transported back to the University of Plymouth, United Kingdom. In the laboratory in Plymouth each sample was then weighed, shredded and rehydrated in a 500ml bottle, containing about 350ml of water. Samples were given approximately 24 hours to soak and were vigorously shaken at least four times during this period and just prior to sieving. The whole bottle contents were washed into two stacked sieves, the top one was a 500µm mesh to collect coarse material and the lower one a 38µm mesh to collect specimens. The top sieve was flushed through with an excess quantity of water to ensure as many specimens were collected as possible. The smaller fraction was then backwashed onto a scored petri dish and immediately searched at a magnification between X20 and X45 using darkfield microscopy. Cleaned specimens were mounted onto microscope slides using Heinze polyvinyl alcohol (PVA).

Specimens were examined in Plymouth using an Olympus BH microscope with

darkfield (cardioid condenser), phase contrast (PhC) or differential interference contrast (DIC); digital images were taken using a Leica DMR microscope with phase contrast; and additionally observations made using an Olympus BH2 phase contrast/DIC microscope courtesy of Professor Diane Nelson at East Tennessee State University. Morphometric measurements were made using an Image Pro Plus© system attached to the Leica DMR microscope.

Body length measurements excluded the fourth pair of legs; the anterior point for measuring the buccal tube was the top of the stylet sheaths; the internal buccal tube width was measured at the level of the insertion of the stylet supports. Claws' measurements were according to Pilato *et al.* (2002) but because the specimen orientations were less than optimal additional measurement for less well orientated claws are also included for future comparisons. The *pt* ratio is the ratio of a measurement compared with the length of the buccal tube (*s. str.*), expressed as a percentage (Pilato 1981).

Class: Eutardigrada Marcus, 1927

Order: Parachela Schuster, Nelson, Grigarick and Christenberry, 1980

Family: Hypsibiidae Pilato, 1969

Sub-family: Itaquasconinae Pilato, 1969

Genus: *Platicrista* Pilato, 1987

***Platicrista ramsayi* sp. nov. (Figs. 3–12)**

Description

The body length of the holotype specimen (Fig. 3) is 319 µm and the paratypes are 313 µm and 565 µm. The body tapers anteriorly from the second pair of legs. The body is transparent or white. The cuticle is smooth and without pores; on one paratype there is a small amount of very tiny granulation on the dorsal cuticle, posterior to the third pair of legs. Eye spots are absent or had dissolved. The specimens were not viewed before placing them in the mounting media which can dissolve some eyespots. The mouth is situated in a ventral/sub-terminal position. Peribuccal lamellae or papillae are not evident. No mucrons or transverse bars or other sculpturing is evident in the buccal aperture. The apophysis for the insertion of the stylet muscles is a broad flat ridge. The buccal apparatus consists of a buccopharyngeal tube, pharyngeal bulb and two macroplacoids. The buccal tube (*s. str.*) (Fig. 11) is rigid, measuring 26.2 µm long; the width 5.2 µm. The *pt* ratio for the insertion of the stylet supports along the buccal tube (*s. str.*) is 98.1%. There is no drop-shaped structure (apodeme) at the transition point to the pharyngeal tube. The pharyngeal tube (Fig. 12) is flexible but only lightly curved in all specimens. It has very fine spiral annulations along its entire length; each line of the annulations is about 0.4 to 0.5 µm

thick. At the posterior end of the pharyngeal tube the aperture is tri-radial, as denoted by three triangular points measuring 1.4 μm high. The pharyngeal tube is 24.9 μm long. The *pt* ratio for the buccopharyngeal tube length is 195%. The furcae of the stylets are modified as in other species within the genus *Platicrista*. The pharyngeal bulb is an elongated bulbous shape; it is not visible in the holotype, but measured 31.0 μm long by 17.0 μm wide in the paratype that measured 313 μm long (Fig. 7). It contains two very thin macroplacoid sets. The first macroplacoids measures 5.4 μm long; the second positioned close to the first were 15.8 μm long. Pharyngeal apophysis, microplacoid and septulum are all absent.

The claws on each leg are asymmetrical double-claws of a *Hypsibius*-type, i.e. the order of the claw branches with respect to the frontal plane of the legs is primary-secondary-primary-secondary (1212). The external double-claw (Fig. 9) is larger than the internal (Fig. 8); with a larger, straighter primary branch. The primary branch on each double-claw has two small accessory points. On leg pairs I to III, the base of the internal double-claws are without lunules and are not expanded. On the external double-claws the bases are slightly expanded but smooth and without lunules. There is a short, oblique cuticular bar originating near the base of the internal double-claw of leg pairs I to III, (Figs. 4–6, 8). No bar is present between the bases of the internal and external double claws on each leg. The claws on leg pair four (Fig. 10) are larger and each had a smooth expanded base; in particular the expanded base of the posterior double claws is very wide, about 7.5 μm across. The double claws on leg pair four are without lunules or additional cuticular bars. Measurements of some structures of the holotype and the two paratypes are shown in Table 1.

Type material

The holotype is deposited in the Natural History Museum, London, U.K.; registration number 2005:2546. Two paratypes were also found at the same sample site and are maintained in the author's collection; both are very similar to the holotype. Paratype 1 (Figs. 4–7) is similarly orientated as the holotype specimen. Paratype 2 is laterally positioned and either in the late stages of moulting or was a late stage white (type 1) cyst. Paratypes 1 and 2 were from the same moss sample (sample b); the holotype was from another moss sample collected at the same site (sample m).

Remarks

Because only three specimens were recovered from the samples, these were all mounted on to microscope slides. Consequently it was not possible to make any observations of the buccal aperture or buccal armature using SEM techniques. From LM examinations there was no evidence of peribuccal structures such as lamellae, which matches the accepted descriptions for the genus.

Both the holotype and paratype 1 specimens were mounted in an approximate dorsal / ventral orientation which is unsuitable for describing the profile of the buccal apophysis, at the very top of the buccal tube (*s. str.*). In these specimen it is only possibly to describe the

TABLE 1. Summary of measurements for *Platicrista ramsayi* sp. nov.

Character	holotype		paratype 1		paratype 2	
	µm	pt	µm	pt	µm	pt
body length	319.0	1217.6	313.0	1455.8	565.0	1582.6
buccopharyngeal tube length	51.1	195.0	37.2	173.0	68.3	191.3
buccal tube length	26.2		21.5		35.7	
pharyngeal tube length	24.9	95.0	15.7	73.0	32.6	91.3
stylet support insertion point	25.7	98.1	20.6	95.8	34.2	95.8
buccal tube width	5.2	19.8	3.4	15.8	7.6	21.4
Semi lunar structures at the end of the pharyngeal tube	1.4	5.3	1.6	7.4	2.7	7.6
1st macroplacoid length	5.4	20.6	4.3	20.0	11.3	31.7
2nd macroplacoid length	15.8	60.3	13.0	60.5	28.4	79.6
macroplacoid row length	22.1	84.4	18.0	83.7	41.3	115.7
pharyngeal bulb length	-	-	31.0	144.2	-	-
pharyngeal bulb width	-	-	17.0	79.1	-	-
leg 1 external claw primary branch	12.9*	49.2	12.0	55.8	-	-
leg 1 external claw secondary branch	8.4*	32.1	7.1	33.0	-	-
leg 1 internal claw primary branch	10.5*	40.1	9.9	46.0	-	-
leg I oblique cuticular bar length	5.6	21.4	5.3	24.6	-	-
leg 2 external claw primary branch	15.2*	58.0	12.5	81.4	22.1	61.9
leg 2 external claw secondary branch	8.8*	33.6	8.0	37.2	15.2	42.6
leg 2 internal claw primary branch	11.7*	44.7	11.2	52.1	-	-
leg 2 oblique cuticular bar length	5.4	20.6	5.3	24.6	-	-
leg 3 external claw primary branch	14.5	55.3	11.9	55.1	25.1	53.0
leg 3 external claw secondary branch	8.4	32.1	8.3	38.6	15.6	43.7
leg 3 internal claw primary branch	10.1*	38.5	6.4*	29.8	-	-
leg 3 oblique cuticular bar length	5.4	20.6	4.8	22.3	-	-
posterior claw 4 primary branch	17.2	65.6	14.2*	66.0	27.3	76.5
posterior claw 4 secondary branch	11.2	42.7	11.4*	53.0	16.8	47.1
anterior claw 4 primary branch	12.6	48.1	14.8*	68.8	18.4	51.5

* claw in less than ideal orientation

- unsuitable for measurement

buccal apophysis as being very wide, without any obvious crests or an inverted Y-shape process seen in many other Hypsibiidae taxa. Therefore this character could only be examined in a lateral profile on the second paratype specimen. In that specimen it appeared as a shallow flattened ridge, without a distinctive raised shape. However, it must

also be noted that paratype 2 is not in an ideal life history stage for making a detailed description.

Other species of this genus are known to deposit smooth un-sculptured eggs inside the cast exuvia but no specimens which could be attributed to this new species were found.

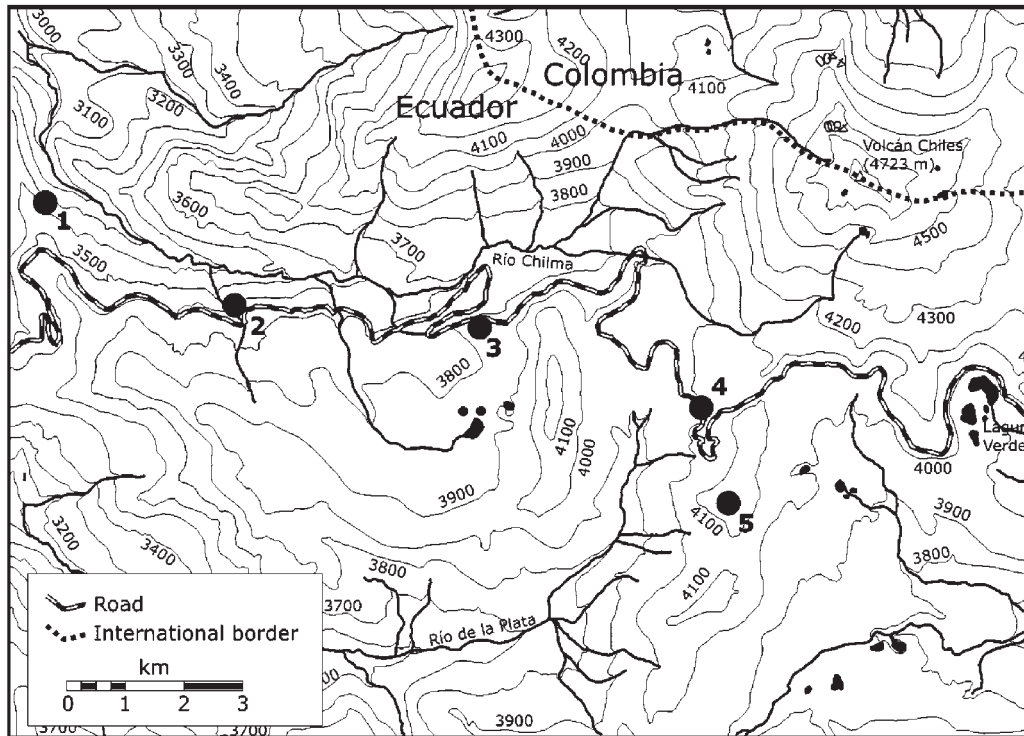


FIGURE 2. Simplified topographical map with the collecting stations (numbered 1–5) for the broader study. Map produced by Dr. Paul Ramsay.

Type locality

The specimens were collected from two unidentified ground moss samples, collected in August 1999 from Volcán Chiles, Ecuador at an altitude of 3,340 m. (Figure 2, station 1)

Etymology

The species is named for Dr. Paul Ramsay, University of Plymouth, for his work on high altitude ecology in the Andes, and for arranging the collecting of the sample materials for the author's tardigrade research from many countries.

Differential diagnosis

Only one other species of the genus *Platicrista* has been described with cuticular bars on its legs, *Platicrista cheleusis* Kathman, 1990. In the original description of *P. cheleusis* (Kathman 1990) and an additional paper by Kathman & Beasley (1997), the bars on the claws can be summarised as follows: on legs I–III there is a small bar between the bases of the internal and external double claws, there is also an oblique bar originating near the

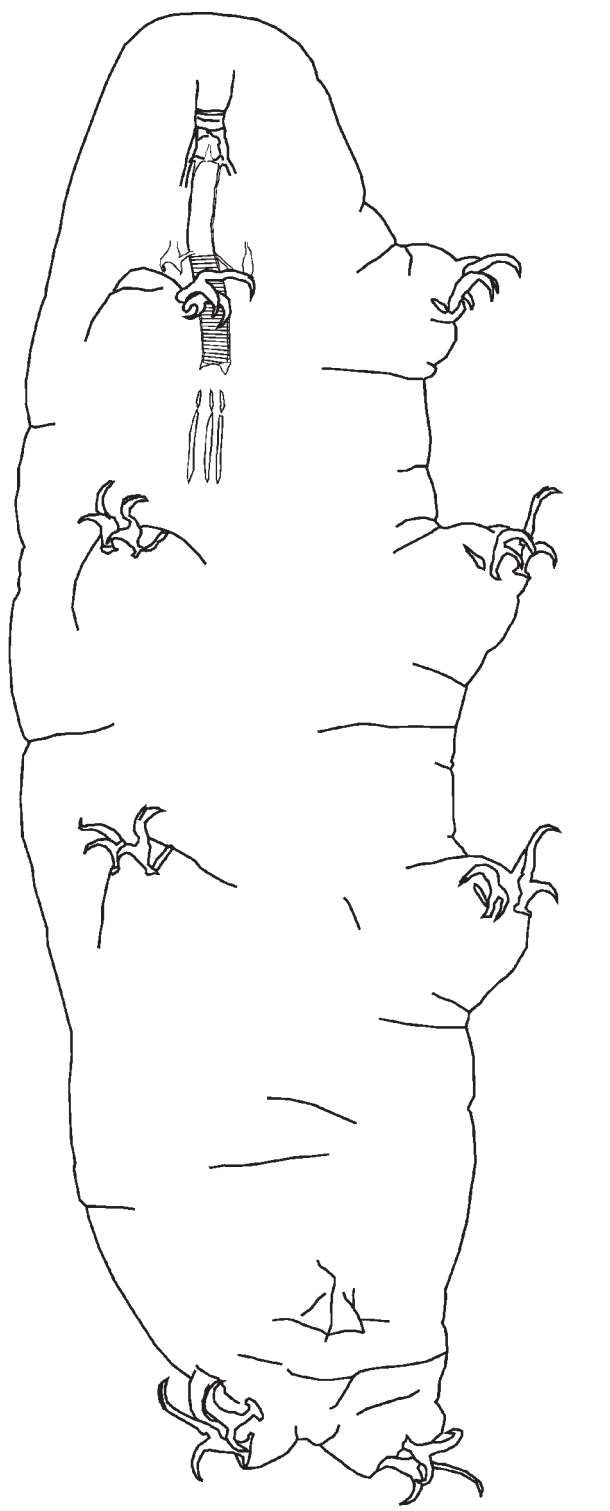
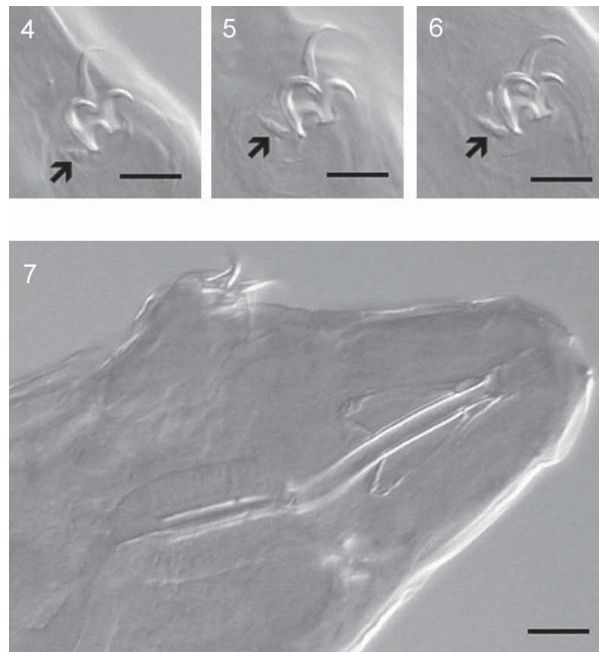


FIGURE 3. Holotype, *Platicrista ramsayi* **sp. nov.**, (drawn from phase contrast microscope; PhC). Scale bar = 100 μ m.

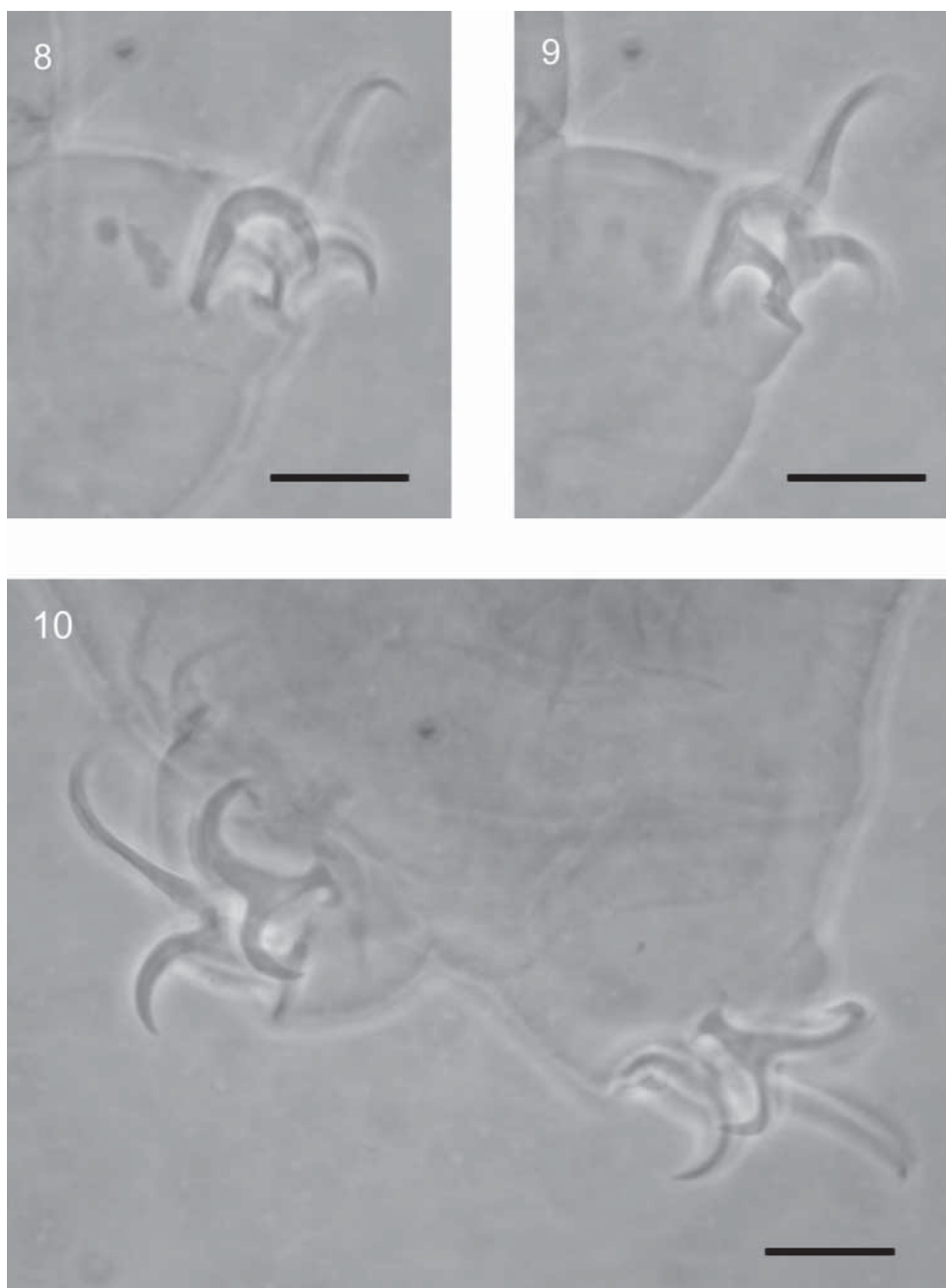


FIGURES 4–7. Paratype 1, *Platicrista ramsayi* **sp. nov.**, 4–6), claws of leg pairs I–III respectively showing the oblique cuticular bars; 7) buccopharyngeal apparatus. DIC, scale bars 4–7 = 10µm.

base of the internal double claw; on the fourth pair of legs there is a small bar between the internal and external double claws only. In *P. ramsayi* cuticular bars are only found on legs I–III and these are only the oblique bars originating near the bases on the internal double claws. Bars between the bases of the internal and external double claws on legs I–IV are absent in *P. ramsayi*. *Platicrista cheleusis* is also described as having a sculptured cuticle with some minor variability reported between the described populations in British Columbia, Canada and Colorado, USA. In *P. ramsayi* the cuticle is smooth, or with just a small amount of very tiny granulation on the dorsal cuticle, posterior to the third pair of legs.

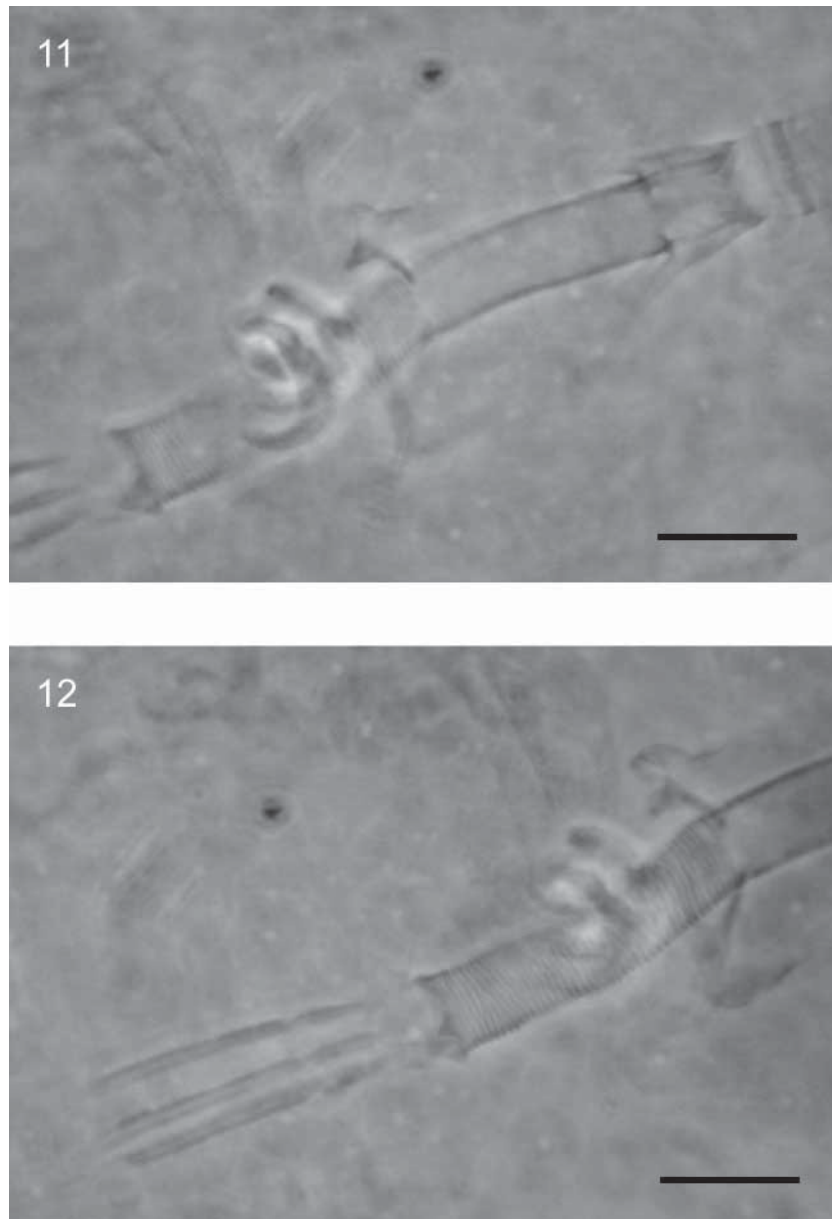
Cuticular bars are absent from the legs of all other species of the genus. *Platicrista angustata* (Murray, 1905) is a well described species from Scotland and is commonly reported in Europe. It is certainly a Holarctic species with additionally specimens collected by the author from localities within the Russian arctic islands. Citations of this species outside the Holarctic should probably be critically re-examined in comparison with specimens from near the type locality, Scotland, since these are mainly very historic records. It does not have cuticular bars on any of its legs.

Platicrista horribilis Kaczmarek & Michalczyk, 2003 is described from Northern Mongolia, and has teeth on the lunules of the claws of leg pair VI which distinguishes it from all other species in the genus. The claws on the fourth pair of legs of the *P. ramsayi* specimens had the bases expanded laterally and were smooth; without teeth or lunules which is the norm for all species within the genus, other than *P. horribilis*.



FIGURES 8–10. Holotype of *Platicrista ramsayi* **sp. nov.** 8–9) claws on leg III with the oblique cuticular bar; 10) claws of leg IV showing the smooth expanded bases without lunules. PhC, scale bars = 10 μm.

Platicrista itaquasconoide (Durante & Maucci, 1975) has a small additional basal spur on the posterior claw on the fourth pair of legs which is absent from all other *Platicrista* species.



FIGURES 11–12. Holotype of *Platicrista ramsayi* **sp. nov.** 11) buccopharyngeal tube, 12) spiral annulations on the pharyngeal tube and the thin sets of placoids. PhC, scale bars = 10µm.

Taxonomic clarifications

When the genus *Platicrista* was first designated by Pilato (1987), two species were formally transferred from the genus *Diphascon* into it, *P. angustata* and *P. itaquasconoide*. Additionally *Platicrista affine* was conditionally transferred into the genus based on its original description and because no specimens were available to examine.

Since then the taxon *P. affine* has been a lingering problem that has been included in

several recent papers describing new species of *Platicrista*, primarily just to be as inclusive and thorough as possible, for which the respective authors can be commended. However, that species has a very brief description, with illustrations of the anterior buccopharyngeal apparatus that are contradictory in their detail; (see Figs. 98a and b in Ramazzotti & Maucci 1983). Additionally the distribution of the macroplacoid sets, in a diamond shape, is a totally atypical condition within all other taxa of the Itaquasconinae, where typically macroplacoids are tightly in parallel. With respect to the legs, cuticular bars would be absent according to the brief description and figures for *P. affine*, thereby differentiating it from *P. ramsayi*. Until such time as another specimen of *P. affine* could be collected and redescribed, this author considers this taxon as a *nomen dubium*, in line with the broader statement made in Dastych (1993) about the numerous taxa which were poorly described by Mihelčič and for which the type material has been destroyed by a flood. This taxon is therefore also omitted from the following key to the genus.

Returning to consider *P. itaquasconoide*, this taxon has only been reported from its type locality and the type material had not been re-examined for some time. Therefore recent authors appear to have relied solely on the description given in Ramazzotti & Maucci (1983, pp. 286–288), a volume which is regarded generally as an accurate compilation of accepted species descriptions up to its date of publication. In that given description the significant diagnostic characters for distinguishing this taxon from its congeners are firstly an additional spur on the base of the posterior claws of leg IV, and secondly the presence of a microplacoid. Both of these characters are clearly illustrated in Ramazzotti & Maucci (1983, Fig. 121) and these figures match the given description.

This author found the presence of the microplacoid a concern when considering this species because it is a character not reported in any other species within the genus. Likewise the modified claw structure sounded significant and a combination of both characters might be enough to warrant considering if this species was in an appropriate genus, *i.e.* should it be transferred into a new genus. The only recourse to settle these questions was to re-examine the holotype specimen, slide number C.T: 3552, maintained in Museum of Natural History in Verona, Italy. The author is therefore grateful to one of the referees for examining the specimen. They confirmed the presence of the additional basal spur on the posterior claws of leg IV. However, there was no indication of a microplacoid.

Two possibilities therefore exist which cannot currently be further investigated without fresh material. Firstly it is possible that a microplacoid was originally present but that it has dissolved or disappeared from sight in this specimen. Hypothetically this might possibly be due to a reaction with the mountant or similar, but I consider this unlikely if the thin macroplacoids were still visible, the microplacoid originally illustrated being quite robust. The second possibility is that the microplacoid illustrated and described is a mistake. Both scenarios are difficult to consider when the original authors were so competent, but the current appearance of the holotype is inconsistent with the accepted description.

At this time I consider the most expedient solution is to amend the current description for *Platicrista itaquasconoide*, so that it does not have a microplacoid. Therefore its distinguishing character from its congeners is the additional spur on the basal section of the posterior claws of leg IV.

Key to the species of the genus *Platicrista*

1. With additional cuticular bar on legs..... 2
- Without additional cuticular bars on legs 3
2. Legs I–III with an oblique cuticular bar on the internal side of the leg from near the base of the internal claw. All legs with a small transverse cuticular bar, positioned between the base of the internal and external claws, but not touching the bases. Cuticle with polygonal pattern.....*Platicrista cheleusis*
- Legs I–III with oblique cuticular bar on the internal side of the leg from near the base of the internal claw. No small transverse cuticular bar present positioned between bases of the internal and external claws on any of the legs. Cuticle without polygonal pattern..... *Platicrista ramsayi*
3. Claws on leg IV with an additional small basal spur*Platicrista itaquasconoide*
- Claws on leg IV without an additional small basal spur 4
4. Claws on leg IV with dentate lunules..... *Platicrista horribilis*
- Claws on leg IV without lunules but with smooth expanded bases
..... *Platicrista angustata*

From a biogeographical viewpoint the distribution of verifiable *Platicrista* records is currently demonstrating a clear difference between each of the species, most taxa appear to be allopatric: *P. angustata* a strong European element recorded in many European localities but notably not yet on the Iberian Peninsula, also an Holarctic element with records for example in Siberia (Biserov 1996), Japan (Ito 1995), New Brunswick, Canada (Argue 1971) and Greenland (Petersen 1951); *P. cheleusis* Vancouver Island, Canada (Kathman 1990) and Colorado, USA (Beasley 1990); *P. horribilis* Mongolia (Kaczmarek and Michalczyk 2003); *P. itaquasconoide* Sweden only (Durrante Pasa and Maucci 1975), a second record in Fauna Europaea for the British Isles cannot be verified because it has no cited reference for its source, probably this second record is a minor data error in the huge data set that was submitted into the Fauna Europaea project; *P. ramsayi* **sp. nov.** Ecuador. Only *P. angustata* and *P. itaquasconoide* currently have overlapping distributions, with the later species only recorded from its type locality. All locations probably had acidic conditions, *i.e.* soil type, base rock, leaf litter, coniferous forests, *etc.* and in micro-environments which rarely dry out.

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